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Stephen Paul Mahinka
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BY HAND

July 30, 1999

Office of Special Nutritionals (HFS-450)
Center for Food Safety and
Applied Nutrition, Food and Drug Administration
200 C St., S.W.
Washington, DC 20204

Dear Sir or Madam:

This notification is being filed pursuant to section 403(r)(6) of the Federal Food, Drug and Cosmetic Act ("FFDCA"), 21 U.S.C. § 343(r)(6), and in accordance with the requirements of 21 C.F.R.§ 101.93. Uniweal, Ltd. of 3 Jupiter Street, North Point, Hong Kong, People's Republic of China, within the past 30 days commenced or intends to commence marketing a dietary supplement bearing the following statements on the label and/or in the labeling:

Name of supplement: CIBONNA™

Dietary ingredients: below — Enzyme Digest (Dried) (a proprietary blend containing the components)

a. Chinese Mahonia (*Mahonia fortunei*, (Lindl) Fedde (in Bot. Jahnl. 31:130. 1901), leaf)

b. Sichuan Teasel (Xu-Duan) (root)

c. Garden Balsam (*Impatiens balsamina L.*, author not identified (in Species Plantasum ed. 1:938 (1753), herb)

d. Cnidium (She-Chuang-Zi) (fruit)

97S-0162

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e. Scurfy-Pea (Bu-Gu-Zhi) (fruit)

f. Dong-Quai (root)

g. Epimedium (herb)

h. Drynaria (Gu-Sui-Bu) (root)

Structure/function statements:

1. This product helps build strong bones—This statement is the subject of the supplement as a whole.

2. This product helps delay the human aging process—This statement is the subject of the supplement as a whole.

Summary of Substantiation:

The claims "helps delay the human aging process" and "helps builds strong bones" for CIBONNA™ are based on, and supported by, clinical and animal testing conducted by Uniweal, Ltd. and/or its cooperators (see the attached reports: (1) Clinical Tests of the Effects of CIBONNA™ on Post-menopausal Osteoporosis (Attachment 1); (2) Clinical Tests of the Effects of CIBONNA™ on Men With Osteoporosis (Attachment 2); and (3) Pre-clinical Tests of the Effects of CIBONNA™ on Helping Build Strong Bone and Helping Delay Aging Process (Attachment 3)).

CIBONNA™ has been demonstrated to help delay the aging process and build strong bones in humans (see Attachments 1 and 2). A 1995-96 clinical study involving 589 female subjects revealed a statistically significant increase in fasting serum alkaline phosphatase ("AKP") among subjects taking CIBONNA™ at both three and six months, compared to subjects in two different control groups (i.e., subjects taking LMZGCJ and estrogen, respectively) ($p<.05$). In addition, the AKP levels in female subjects taking CIBONNA™ at three and six months were significantly greater than the AKP level before use, respectively ($p<.05$). An increase in AKP is indicative of an increase in metabolic activities of collagenocytes and the strengthening of the regeneration function.

In this study of females, the subjects taking CIBONNA™ had statistically significant increases in bone mineral content (BMC), bone width (BW), and BMC/BW after three and six months of use from baseline, both compared to baseline and the two control groups ($p<.01$), while the two control groups showed no statistically significant increases in any of these measures at either three or six months. BMC for these subjects increased 11.28% and 28.1% after CIBONNA™ was taken for three and six months, respectively.

Likewise, a 1996-97 clinical study involving 496 male subjects revealed a statistically significant increase in fasting serum alkaline phosphatase ("AKP") among male subjects taking CIBONNA™ at both three and six months, compared to subjects in a control group (i.e., subjects taking LMZGCJ) ($p<.05$). In addition, the AKP levels in male subjects taking CIBONNA™ at three and six months were significantly greater than the AKP level before use ($p<.05$).

In this study of males, the subjects taking CIBONNA™ had statistically significant increases in bone mineral density (BMD) at the positions of lumbar vertebra (L_{2-4}) and the upper portion of thigh bone (Neck, Ward's, and Troch) after three and six months of use from baseline, both compared to baseline and the control group ($p<.01$), while the control group showed no statistically significant increases in any of these measures at either three or six months.

Therefore, based on the above study results, CIBONNA™ has been demonstrated in humans to help delay the aging process, and to help build strong bones by reducing bone loss and increasing bone mineral content.

The above results are further substantiated by the results of Uniweal Ltd.'s animal studies. A 1995-98 rat study revealed that the tensile strength of collagen fibers of the caudal tendon of rats injected with CIBONNA™ was virtually unchanged for rats of different ages, while rats who were not treated with CIBONNA™ showed an exponential correlation between the tensile strength of collagen fibers of the caudal tendon with the ages of rats ($p<.01$). Because the percentage of extracted collagen from the skins of rats decreases as rats become older ($p<.01$), the above result demonstrates that CIBONNA™ helps delay the aging process in rats.

Further, the animal study revealed that rats injected with CIBONNA™ exhibited unchanged BMC as they got older, while rats who were not treated with CIBONNA™ showed a rapid decrease in BMC as they got older ($p<.01$). Because left untreated, the BMC in rats decreases with age, CIBONNA™ builds strong bones in rats. Biomechanical testing showed that modulus elasticity ($p<.01$), flexibility strength ($p<.01$), destructibility strength ($p<.01$), flexibility enthalpy ($p<.01$), and destructibility enthalpy ($p<.01$) were greater in rats treated with CIBONNA™ compared to those who were not.

For more details concerning the above results, including a description of the testing methodologies and an explanation of the biomechanical tests, see the attached reports.

The undersigned certifies that the information presented and contained in this notification is complete and accurate, and that Uniweal, Ltd. has substantiation that each structure/function statement is truthful and not misleading.

Sincerely,

Stephen Paul Mahinka
Counsel for Uniweal, Ltd.

Attachments

Attachment 1

Clinical Tests of the Effects of CIBONNA™ on Post-menopausal Osteoporosis

I. Methods

Clinical testing of CIBONNA™ in female humans was conducted between March 1995 and May 1996. This testing involved 589 female volunteers with post-menopausal osteoporosis (PMO) (age 49 to 75 years) with an average age of 63.7± 8.9 years old (X ± S; same syntax used below). The volunteers weighed between 43 - 85kg, and the average duration of menopause was 12.3 ± 7.6 years.

None of the volunteers regularly or routinely smoked, drank alcohol, had endocrinopathy or other serious chronic diseases, or abnormal heart, liver or kidney function. For at least three months prior to the tests, none of the volunteers took estrogen, calcitonin and any other drugs that have an effect on bone metabolism except for calcium supplements. Volunteers were also excluded on the basis of secondary osteoporosis caused by diseases such as hyperthyroidism, diabetes, rheumatoid arthritis and multiple myeloma, and other serious complications.

The selected volunteers were randomly divided into three groups: (1) 389 cases in the CIBONNA™ group; (2) 100 cases in the LMZGCJ[1/] control group; (3) and 100 cases in the estrogen control group. There were no statistically significant differences between these three groups in terms of physical/medical history characteristics (i.e., age, weight, post-menopausal time).[2/]

All volunteers were diagnosed according to the Score Index[3/] for PMO's Comprehensive Analysis-Diagnosis.[4/] All volunteers' scores were above 5, and the scores of the three groups are listed in Table 1.

1/ LMZGCJ is primarily composed of Os Draconis, Concha Ostreae, Rhizoma Atractylodis Macrocephalae, Radix Astragali, and Carpax et Plastrum Testudinis.

2/ When statistical *in*significance is reported in this study report, such a finding is based on a 95% confidence interval using a t-test statistic, i.e., p>0.05 based on a t-test.

3/ See World Health Organization, Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis (Report of a WHO Study Group), Technical Report Series, No. 843 (1994); World Health Organization, Guidelines for Preclinical Evaluation and Clinical Trials in Osteoporosis (1998).

4/ See Osteoporosis 170 (Zhonghou Liu ed., Chemical Industry Publishing House 1st ed. 1992).

In the CIBONNA™ group, researchers administered CIBONNA™ to the volunteers orally in the following dosage: two capsules, 4 times per day. One capsule contains 200 mg of CIBONNA™. Volunteers took their doses one half-hour before breakfast, lunch, supper, and bed time, respectively, with warm boiled water.

In the LMZGCJ control group, the volunteers orally took LMZGCJ produced by Wuhan Jianmin Pharmaceutical Factory (lot number: 930302; Contents: 5g/bag) in the following doses: two bags, 4 times per day. Volunteers took their doses of LMZGCJ on the same schedule as the CIBONNA™ group.

In the estrogen control group, the volunteers took orally diethyl stilbestrone produced by Shanghai Squibb Company (lot number: 941102; contents: 15mg/tablet) at a dose of one tablet, two times per day.

II. Findings

A. Measurement of Bone Mineral Density

Using single photon absorptionmetry (Model SP-200, produced by Beijing Geology Institute), the non-dominant side upper limb of the volunteers were measured for bone mineral density by the method of x-ray absorption. The measuring point was selected as the 1/3 boundary at the center and lower of radius, and the measuring scope was at the measuring point ± 0.5cm. Parameters were automatically printed-out via a computer readout, including bone mineral content (BMC), bone width (BW), and BMC/BW.

Measurements of BMC, BW, and BMC/BW were made at baseline (i.e., before volunteers began taking CIBONNA™), and at three and six months after the CIBONNA™ group started taking CIBONNA™. The CIBONNA™ group had statistically significant increases in BMC, BM, and BMC/BM after three and six months of use from baseline, both compared to baseline and the two control groups ($p<.01$), while the two control groups showed no statistically significant increases in any of these measures at either three or six months.[5/] See Table 2.

Therefore, CIBONNA™ can efficiently help build strong bone by reducing bone loss and increasing bone mineral content. BMC increased 11.28% and 28.1% after CIBONNA™ was taken for three and six months, respectively (see Table 2). This means CIBONNA™ maintains bone mineral density at normal or close to normal levels (i.e. the average bone mineral density is recovered from $\leq X \pm 2S$ to $X \pm 1S$).

5/ Data were processed by the method of matched-pairs, the measurement data were expressed as $X \pm S$, and the differences were assessed by using the method of a Student's t-test.

B. Biochemical Analysis of Bone Metabolism

A biochemical analysis of bone metabolism involved the below steps.

1. Fasting serum alkaline phosphatase (AKP)—Serum was separated from fasting blood and stored at -20 °C. All samples in the same lot were measured at the same time according to "Regulations of Operation for Clinical Test in China." The coefficient of variation in one lot was 1.89%.

2. Ratio of calcium in urine/creatinine (u-ca/er)—The second fasting urine sanguinis was obtained for two consecutive days, and stored at -20 °C. All samples in the same lot were measured at the same time. The urine was thawed before examination. The two days samples were mixed, and then examined. U-Ca was determined by the method of orthocresol complexing ketone, and creatinine was determined by the method of picric acid. The coefficient of variation in one lot was 4.4%.

3. Ratio of Hydroxyproline in urine/creatinine (U-HOP/cr)—The concentration of hydroxyproline of the above-mentioned samples were measured by the method of ammonia-aminate test. The coefficient of variation in one lot was 5.4%.

4. Serum Ca—Serum calcium was determined by using a similar method as calcium in urine was determined. The coefficient of variation in one lot was less than 5%.

5. Serum P—Serum phosphorous was determined by using the method of colorimetry. The coefficient of variation in one lot was 0.75%.

6. Serum glutamate pyruvate transaminase (SGPT)—SGPT was determined by using the method of enzyme hydrolysis rate, and the coefficient of variation in one lot was 3.1%.

Bone metabolism measurements were made at baseline (i.e., before use of CIBONNA™) and at three and six months after use of CIBONNA™. Data were processed by the method of matched-pairs, the measurement data were expressed as $X \pm S$, and the differences were assessed by using the method of a Student's t-test.

As shown in Table 3, the CIBONNA™ group showed statistically significant increases in AKP, Serum P, U-HOP/Cr, and U-Ca/cr after three and six months after use of CIBONNA™ ($p<0.05$), while the same cannot be said for the control group treatments. An increase of AKP translates into an increase of metabolic activities of collagenocytes and the strengthening of the regeneration function. Therefore, CIBONNA™ can help delay the human aging process by improving the activities of collagenocytes.

III. Conclusion

Female subjects taking CIBONNA™ had a statistically significant increase in fasting serum alkaline phosphatase ("AKP") at both three and six months, compared to subjects in two different control groups (i.e., subjects taking LMZGCJ and estrogen, respectively), and the AKP levels in female subjects taking CIBONNA™ at three and six months were significantly greater than the AKP level before use, respectively. An increase in AKP is indicative of an increase in metabolic activities of collagenocytes and the strengthening of the regeneration function.

Female subjects taking CIBONNA™ also had statistically significant increases in bone mineral content (BMC), bone width (BW), and BMC/BW after three and six months of use from baseline, both compared to baseline and the two control groups, while the two control groups showed no statistically significant increases in any of these measures at either three or six months. BMC for these subjects increased 11.28% and 28.1% after CIBONNA™ was taken for three and six months, respectively.

Therefore, based on the above study results, CIBONNA™ has been demonstrated to help delay the aging process, and to help build strong bones by reducing bone loss and increasing bone mineral content, in females.

Table 1 CIBONNA™/PMO Clinical Study: PMO Scores of the Three Female Groups

Group	Number of score 5	Number of score 6	Number of score 7	Number of score 8	Average score*
CIBONNA™	34	58	156	141	7.04
LMZGCJ control group	9	15	41	35	7.02
Estrogen control group	9	15	40	36	7.03

* No significant differences between groups (p>0.05)

A paired t-test statistic was utilized to compare groups.

Table 2 **CIBONNA™/PMO Clinical Study: Bone Mineral Density Before and After Use of CIBONNA™ (X ± S)**

Group	No	Time Period	BMC (g/cm)	BW (cm)	BMC/BW (g/cm^2)
CIBONNA™ group	389	before use	0.633 ± 0.121	1.209 ± 0.163	0.523 ± 0.94
	389	three month	0.712 ± 0.138 ◊★	1.224 ± 0.135 ◊★	0.582 ± 0.097 ◊★
	389	six month	0.837 ± 0.153 ◊★	1.248 ± 0.170 ◊★	0.670 ± 0.100 ◊★
LMZGCJ control group	100	before use	0.678 ± 0.141	1.213 ± 0.148	0.559 ± 0109
	100	three month	0.640 ± 0.138	1.211 ± 0.147	0.551 ± 0.110
	100	six month	0.658 ± 0.134	1.204 ± 0.161	0.547 ± 0.115
Estrogen control group	100	before use	0.649 ± 0.127	1.204 ± 0.135	0.539 ± 0.111
	100	three month	0.644 ± 0.132	1.098 ± 0.143	0.537 ± 0.113
	100	six month	0.647 ± 0.116	1.201 ± 0.139	0.538 ± 0.109

◊ compared to baseline (within group), $p<0.01$
★ compared with other groups, $p<0.01$

A paired t-test statistic was utilized to compare groups (both within-group differences from baseline and between groups).

Table 3 **CIBONNA™/PMO Clinical Study: Changes of Bone Metabolism Before and After Use of CIBONNA™**

Group	Time	Blood AKP	Blood Ca	Blood P	U-Ca/Cr	U-HOP/Cr
CIBONNA™ group (n=389)	before use	52.14 ± 16.22	2.07 ± 0.31	0.95 ± 0.22	0.273 ± 0.184	13.84 ± 6.03
	three month	65.42 ±19.95◇★	2.34 ± 0.26	1.22 ± 0.24◇★	0.539 ± 0.201◇★	16.89 ± 5.51◇★
	six month	66.05 ± 21.53◇★	2.35 ± 0.25	1.21 ± 0.23◇★	0.551 ± 0.237◇★	15.23 ± 6.28◇★
LMZGCJ control group (n=100)	before use	45.47 ± 12.65	2.09 ± 0.34	0.95 ± 0.17	0.289 ± 0.144	13.66 ± 4.52
	three month	48.53 ± 11.38	2.11 ± 0.19	0.98 ± 0.23	0.274 ± 0.158	12.87 ± 6.53
	six month	47.61 ± 12.32	2.06 ± 0.23	0.89 ± 0.25	0.269 ± 0.155	12.45 ± 5.98
Estrogen control group (n=100)	before use	48.44 ± 12.59	2.08 ± 0.30	0.87 ± 0.30	0.265 ± 0.149	13.31 ± 6.23
	three month	46.23 ± 15.38	2.24 ± 0.25	0.92 ± 0.26	0.271 ± 0.154	14.52 ± 6.10
	six month	49.62 ± 13.45	2.16 ± 0.34	0.97 ± 0.24	0.284 ± 0.146	12.89 ± 4.97

◇ compared to baseline (within group), $p<0.05$

★ compared with other groups, $p<0.05$

The CIBONNA™ group showed statistically significant increases in AKP, Serum P, U-HOP/Cr, and U-Ca/cr ($p<0.05$) after three and six months after use of CIBONNA™, while the same cannot be said for the control group treatments.

A paired t-test statistic was utilized to compare groups (both within-group differences from baseline and between groups).

Attachment 2

Clinical Tests of the Effects of CIBONNA™ on Men With Osteoporosis

I. Methods

Clinical testing of CIBONNA™ in male humans was conducted between January 1996 and August 1997. This testing involved 496 male volunteers with osteoporosis (OP), age 60 to 89. The average age of the volunteers was 71.4 ± 12.1 years old (X ± S; same syntax used below), and the volunteers weighed between 58-87 kg. None of the volunteers had endocrinopathy, other serious chronic diseases, or abnormal heart, liver or kidney function. For three months prior to the testing, none of the volunteers received estrogen, calcitonin and any other drugs that have an effect on bone metabolism except for calcium supplements. Volunteers were also excluded on the basis of secondary osteoporosis associated with diseases such as hyperthyroidism, diabetes, rheumatoid arthritis, and multiple myeloma, as well as volunteers with serious complications. Therefore, the study only included volunteers with OP due to aging.

The selected volunteers were randomly divided into the following groups: (1) 381 cases into the CIBONNA™ group; and (2) 115 cases into the LMZGCJ[1/] control group. There were no statistically significant differences between these two groups in terms of physical characteristics (e.g., age, weight).[2/] All volunteers were diagnosed according to the Score Index[3/] for PMO's Comprehensive Analysis-Diagnosis,[4/] and all volunteers' scores were above 5 (see Table 1).

In the CIBONNA™ group, the volunteers orally took CIBONNA™ at the following dose: two capsules, 4 times per day. One capsule contained 150 mg of CIBONNA™. The volunteers took the CIBONNA™ doses one-half hour before breakfast, lunch, supper, and bed time with warm boiled water.

In the LMZGCJ control group, the volunteers orally took LMZGCJ produced by Wuhan Jianmin

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1/ LMZGCJ is primarily composed of Os Draconis, Concha Ostreae, Rhizoma Atractylodis Macrocephalae, Radix Astragali, and Carpax et Plastrum Testudinis.

2/ When statistical *in*significance is reported in this study report, such a finding is based on a 95% confidence interval using a t-test statistic, i.e., p>0.05 based on a t-test.

3/ See World Health Organization, Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis (Report of a WHO Study Group), Technical Report Series, No. 843 (1994); World Health Organization, Guidelines for Preclinical Evaluation and Clinical Trials in Osteoporosis (1998).

4/ See Osteoporosis 170 (Zhonghou Liu ed., Chemical Industry Publishing House 1st ed. 1992).

Pharmaceutical Factory (lot number: 951103; contents: 5g/bag) at the following dose: two bags, 4 times per day. This group followed the same schedule for administration as the CIBONNA™ group.

II. Findings

A. Measurement of Bone Mineral Density

Using a DPX-L dual energy x-ray bone density apparatus (produced by Lunar, a US company), bone mineral density (BMD) of the volunteers was measured at the positions of lumbar vertebra ($L_{2\text{-}4}$ and the upper portion of thigh bone (Neck, Ward's, Troch). The apparatus was controlled by a computer and the data were analyzed automatically and the results printed-out. The apparatus was tested before measurements were taken each day, pursuant to the standard and routine procedure for testing this apparatus. The standard of OP diagnosis was based on subtracting two standard deviations (SD) from the peak values of BMD at the same position and with the same sex.

Measurements of the BMD of $L_{2\text{-}4}$, Neck, Ward's, and Troch were made at baseline (i.e., before volunteers began taking CIBONNA™), and at three and six months after the CIBONNA™ group started taking CIBONNA™. The CIBONNA™ group had statistically significant increases in BMD of $L_{2\text{-}4}$, Neck, Ward's, and Troch after three and six months of use from baseline, both compared to baseline and the control group ($p<.01$), while the control group showed no statistically significant increases in any of these measures at either three or six months.[5/] See Table 2. Therefore, CIBONNA™ can efficiently help build strong bone by reducing bone loss and increasing bone mineral content. This means CIBONNA™ maintains bone mineral density at normal levels (i.e., the average bone mineral density is recovered from $\leq X \pm 2S$ to $X \pm 1S$).

B. Biochemical Analysis of Bone Metabolism

A biochemical analysis of bone metabolism involved the below steps.

1. Fasting serum alkaline phosphatase (AKP)—Serum was separated from fasting blood and stored at -20 °C. All samples in the same lot were measured at the same time according to "Regulations of Operation for Clinical Test in China", and the coefficient of variation in one lot was 1.89%.

2. Ratio of calcium in urine/creatinine (u-ca/er)—The second fasting urine sanguinis was

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5/ Data were processed by the method of matched-pairs, the measurement data were expressed as $X \pm S$, and the differences were assessed by using the method of a Student's t-test.

obtained for two consecutive days, and stored at -20 °C. All samples in the same lot were measured at the same time. The urine was thawed before examination. The two days samples were mixed, and then examined. U-Ca was determined by the method of orthocresol complexing ketone, and creatinine was determined by the method of picric acid. The coefficient of variation in one lot was 4.4%.

3. Ratio of Hydroxyproline in urine/creatinine (U-HOP/cr)—The concentration of hydroxyproline of the above-mentioned samples were measured by the method of ammonia-aminate test. The coefficient of variation in one lot was 5.4%.

4. Serum Ca—Serum calcium was determined by using a similar method as calcium in urine was determined. The coefficient of variation in one lot was less than 5%.

5. Serum P—Serum phosphorous was determined by using the method of colorimetry. The coefficient of variation in one lot was 0.75%.

6. Serum glutamate pyruvate transaminase (SGPT)—SGPT was determined by using the method of enzyme hydrolysis rate, and the coefficient of variation in one lot was 3.1%.

Measurements were made before use of CIBONNA™, and at three and six month after use of CIBONNA™, respectively (see Table 3). Data were processed by the method of matched-pairs, the measurement data were expressed as $X \pm S$, and the differences were assessed by using the method of a Student's t-test.

As shown in Table 3, the CIBONNA™ group showed statistically significant increases in AKP, Serum P, U-HOP/Cr, and U-Ca/cr after three and six months after use of CIBONNA™ ($p<0.05$), while the same cannot be said for the control group treatment. An increase of AKP translates into an increase of metabolic activities of collagenocytes and the strengthening of the regeneration function. Therefore, CIBONNA™ can help delay the human aging process by improving the activities of collagenocytes.

III. Conclusion

Male subjects receiving CIBONNA™ had a statistically significant increase in fasting serum alkaline phosphatase ("AKP") among male subjects taking CIBONNA™ at both three and six months, compared to subjects in a control group (i.e., subjects taking LMZGCJ), and AKP levels in male subjects taking CIBONNA™ at three and six months were significantly greater than the AKP level before use ($p<.05$). An increase in AKP is indicative of an increase in metabolic activities of collagenocytes and the strengthening of the regeneration function.

Male subjects taking CIBONNA™ also had statistically significant increases in bone mineral density (BMD) at the positions of lumbar vertebra ($L_{2\text{-}4}$) and the upper portion of thigh bone (Neck, Ward's, and Troch) after three and six months of use from baseline, both compared to baseline and the control group, while the control group showed no statistically significant increases in any of these measures at either three or six months.

Therefore, based on the above study results, CIBONNA™ has been demonstrated to help delay the aging process, and to help build strong bones by reducing bone loss and increasing bone mineral content, in males.

Table 1 CIBONNA™/Male Clinical Study: PMO Scores of the Two Groups

Group	Number of Score 5	Number of score 6	Number of score 7	Number of score 8	Average score*
CIBONNA™ group	7	117	91	116	7.09
LMZGCJ control group	3	34	28	0	7.08

* No significant differences between groups ($p>0.05$)

A paired t-test statistic was utilized to compare groups.

Table 2 **CIBONNA™/Male Clinical Study: Bone Mineral Density Before and After Use of CIBONNA™ (g/cm^2, $X \pm S$)**

Group	No	Time Period	$L_{2\text{-}4}$	Neck	Ward's	Troch
CIBONNA™ group	381	before use	0.843 ± 0.148	0.795 ± 0.131	0.659 ± 0.153	0.736 ± 0.135
	381	three month	1.047 ± 0.150 ◇★	0.855 ± 0.149 ◇★	0.757 ± 0.174 ◇★	0.823 ± 0.111 ◇★
	381	six month	1.195 ± 0.122 ◇★	0.974 ± 0.122 ◇★	0.842 ± 0.177 ◇★	0.857 ± 0.094 ◇★
LMZGCJ control group	115	before use	0.877 ± 0.139	0.805 ± 0.141	0.661 ± 0.166	0.725 ± 0147
	115	three month	0.879 ± 0.141	0.813 ± 0.155	0.669 ± 0.149	0.731 ± 0.152
	115	six month	0.894 ± 0.137	0.823 ± 0.138	0.667 ± 0.157	0.738 ± 0.161

◇ compared with before use, $p<0.01$
★ compared with other groups, $p<0.01$

A paired t-test statistic was utilized to compare groups; both within-group differences from baseline and between groups

Table 3 CIBONNA™/Male Clinical Study: Changes of Bone Metabolism Before and After Use of CIBONNA™

Group	Time	Blood AKP	Blood Ca	Blood P	U-Ca/Cr	U-HOP/Cr
CIBONNA™ group (n=381)	before use	46.23 ± 13.61	2.14 ± 0.27	0.89 ± 0.21	0.284 ± 0.204	12.17 ± 5.74
	three month	63.11 ± 20.64 ◇★	2.03 ± 0.24	1.18 ± 0.23 ◇★	0.237 ± 0.243 ◇★	17.63 ± 6.11 ◇★
	six month	67.21 ± 17.25 ◇★	2.21 ± 0.19	1.04 ± 0.29 ◇★	0.307 ± 0.169 ◇★	17.05 ± 5.94 ◇★
LMZGCJ control group (n=115)	before use	45.33 ± 11.47	2.11 ± 0.27	0.96 ± 0.21	0.245 ± 0.176	13.11 ± 5.07
	three month	47.64 ± 12.11	2.43 ± 0.25	1.04 ± 0.19	0.293 ± 0.201	13.04 ± 6.72
	six month	46.22 ± 13.06	2.46 ± 0.28	1.01 ± 0.24	0.271 ± 0.175	11.96 ± 5.09

◇ compared to baseline (within group), $p<0.05$
★ compared with other group, $p<0.05$

The CIBONNA™ group showed statistically significant increases in AKP, Serum P, U-HOP/Cr, and U-Ca/cr ($p<0.05$) after three and six months after use of CIBONNA™, while the same cannot be said for the control group treatment.

A paired t-test statistic was utilized to compare groups (both within-group differences from baseline and between groups).

Attachment 3

Pre-clinical Tests of the Effects of CIBONNA™ on Helping Build Strong Bone and Helping Delay Aging Process

I. Methods

Animal testing involving CIBONNA™ was conducted between 1995 and 1998. The comparisons of groups detailed in this study report based on an analysis of variance (ANOVA) method and a Student's t-test between groups, based on a 95% confidence interval (unless stated otherwise).

The animal testing involved 540, six-month old female Wistar rats that weighed between 300g and 350g. The rats were randomly divided into three groups: (1) Group A (Normal); (2) Group B (CIBONNA™); and Group C (Control) (see Table 1). Groups A and C did not differ significantly in their average weight from that of the CIBONNA™ Group (Group B) ($p>.05$).

Table 1. Groups of Rats

Group	Rat	Average Weight
Group A	180	326.36 ± 25.09*
Group B	180	323.92 ± 22.22
Group C	180	328.04 ± 24.79*

*t-test; comparison with the CIBONNA™ group: $p>0.05$

The rats in Groups B and C were ovariectomized by operation and then fed for 42 days with standard feed to build osteoporosis models. Group A was under sham operation without removing the ovaries of the rats. Forty-two days after the operation, all three groups were divided into smaller groups.

Group A was divided into six subgroups randomly with each subgroup having 30 rats (see Table 2). The six subgroups of Group A did not differ significantly in their average weight ($p>.05$).

Table 2. Subgroups of Group A (Normal)

Group	Rat	Average Weight
A1	30	346.17 ± 32.54
A2	30	338.21 ± 29.69
A3	30	335.49 ± 27.68
A4	30	341.52 ± 31.92
A5	30	351.01 ± 32.94
A6	30	343.23 ± 28.63

Group B was divided into six subgroups randomly with each subgroup having 30 rats (see Table 3). Each rat of Group B was injected intraperitoneally with 0.3 mg of CIBONNA™ one time per day (the CIBONNA™ injection was prepared by dissolving CIBONNA™ in sterile double-distilled water at 37 °C for 24 hours, with the volume of the injection being 1 ml of the supernatant containing 0.3 mg of CIBONNA™ per 1 ml). The six subgroups of Group B did not differ significantly in their average weight ($p>.05$).

Table 3. Subgroups of Group B (CIBONNA™)

Group	Rat	Average Weight
B1	30	354.18 ± 37.61
B2	30	357.71 ± 39.54
B3	30	351.82 ± 34.63
B4	30	360.24 ± 41.73
B5	30	358.48 ± 40.01
B6	30	355.49 ± 38.23

Group C was divided into six subgroups randomly with each subgroup having 30 rats (see Table 4). Each rat of Group C was injected intraperitoneally with 1 ml of 0.9% normal saline one time per day. The six subgroups of Group C did not differ significantly in their average weight ($p>.05$).

Table 4. Subgroups of Group C (Control)

Group	Rat	Average Weight
C1	30	361.27 ± 41.23
C2	30	359.48 ± 37.79
C3	30	354.93 ± 38.12
C4	30	357.66 ± 39.25
C5	30	360.11 ± 38.67
C6	30	356.42 ± 40.31

After finishing the above grouping, the rats of each subgroup were anesthetized by injecting intraperitoneally 30 mg/kg.db[1/] of 1% sodium pentobarbital under the time order shown in Table 5.

Table 5. Time/Order of Anesthetizing

Time(week)	Group
0	A1, B1, C1
12	A2, B2, C2
24	A3, B3, C3
36	A4, B4, C4
48	A5, B5, C5
60	A6, B6, C6

From Table 5, it is shown that the higher-number groups are older than the lower-number groups (e.g., the rats in group A2 are older than those in group A1, and the rats in group A4 are older than those in group A3).

1/ "db" means "dropping bottle."

II. Findings

A. Delaying the Aging Process: Assays of Tensile Strength of Collagen Fibers of the Caudal Tendon of Rats

A group of collagen fibers were obtained from the rats by cutting skin of the caudal tendon at the position of 1 to 1.5 cm from the caudal end. The group of collagen fibers were placed in a culture dish containing cold normal saline and separated into individual collagen fiber. The individual fibers were measured for their lengths and diameters. One end of an individual collagen fiber was fixed to a sample hook of an isolated bath and the other end at the position of 3 cm away from the hook was given 2 g of tensile (tensile loading) through a slide car (i.e., a constant load is placed on the fiber). The bath contained 7 mol/1 of urea buffer solution (7 mol/1 urea., 0.06 mol/l KH_2PO_4, 0.02 mol/l $NaBO_3$, pH 7.5) and the temperature was kept at 40°C. The breaking time was recorded for each individual collagen fiber of each group and average breaking time was calculated based on the breaking time of every three individual collagen fibers. The results of each of the three groups is provided in Table 6.

Table 6. Tensile Strength of Collagen

Group	Time of Experiment (day)	Sample	Breaking Time(s)
A1 (Normal)	222 ± 15	30	1927.4 ± 84.6
A2	306 ± 15	30	2541.0 ± 76.7
A3	390 ± 15	30	2925.6 ± 127.2
A4	474 ± 15	30	3594.7 ± 204.3
A5	558 ± 15	30	4594.6 ±246.7
A6	642 ± 15	30	4854.6 ± 298.5
B1 (CIBONNA™)	222 ± 15	30	3121.4 ± 107.9
B2	306 ± 15	30	2754.8 ± 114.6
B3	390 ± 15	30	2414.7 ± 95.3
B4	474 ± 15	30	2501.1 ± 128.5
B5	558 ± 15	30	2463.2 ± 136.4
B6	642 ± 15	30	2549.5 ± 146.3
C1 (Control)	222 ± 15	30	3008.7 ± 134.9
C2	306 ± 15	30	3665.4 ± 209.5
C3	390 ± 15	30	4278.5 ± 305.1
C4	474 ± 15	30	4892.3 ± 394.4
C5	558 ± 15	30	5145.9 ± 474.1
C6	642 ± 15	30	5731.4 ± 503.9

The breaking time results in Table 6 show that the tensile strength of collagen fibers of the caudal tendon for the Normal and Control groups have a positive exponential correlation with the ages of the rats,[2/] while the tensile strength of the CIBONNA™ group remains almost

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2/ The exponential correlation of the breaking time of collagen fibers of the caudal tendon for the Normal group with the ages of rats is as follows: $T_{b,A} = 1.175 \times 10^7 e^{0.0023T_s}$. The (continued...)

unchanged within a period of ages of rats. This shows that CIBONNA™ has an effect on helping delay the rat aging process.[3/]

B. Delaying the Aging Process: Extracting Skin Collagen of Rats

Immediately after the rats were sacrificed, the skins of the rats were pilled off. Hairs and subcutaneous fats were removed from the skins. The skins were cut into pieces with 1 x 1 mm after wiping off fat or oil on the skins. One gram of the skin pieces were placed into a tube, and 20 ml of 0.01 mol/l sodium acetate solution (pH 7.0) was added to the tube. The skin pieces were incubated for 3 hours by placing the tube into a water bath at 80°C. The tube was then cooled in ice and centrifuged at 5000g for 30 minutes. The supernatants and pellets were ice dried and the contents of hydroxproline (HC) were determined by using a method of ammonia-aminate test. The proportion of collagen extracted was based on the following equation:

$$\text{Proportion of collagen extracted} = \frac{\text{HC of the supernatants}}{\text{HC of the supernatants and precipitates}} \times 100\%$$

The proportions of extracted collagen from the skins for each of the three groups are provided in Table 7.

2/(...continued)
exponential correlation of the breaking time of collagen fibers of the caudal tendon for the Control group with the ages of rats is as follows: $T_{b,C} = 6.166 \times 10^7 e^{0.00137Ts}$.

3/ See Everitt AV et. al., Dietary, Caging, And Temperature Factors In The Aging of Collagen Fibres In Rat Tail Tendon, Gerontology 27(1-2):37-41 (1981). It has been reported that stronger tendons have a negative correlation with aging since the 1960s. See e.g., Olsen GG et. al., Retardation of the Ageing Process In Collagen Fibres From The Tail Tendon Of The Old Hypophysectomized Rat, Nature 206(981):307-8 (1965).

Table 7. Results of Collagen Extraction

Group	Sample	Proportion of Collagen Extracted (%)
A1 (Normal)	8	93.7 ± 2.40
A2	8	94.1 ± 2.54
A3	8	79.6 ± 4.53
A4	8	77.8 ± 5.07
A5	8	47.6 ± 5.41
A6	8	43.7 ± 0.53
B1 (CIBONNA™)	8	75.8 ± 7.23
B2	8	91.4 ± 3.22
B3	8	93.5 ± 2.61
B4	8	94.3 ± 2.16
B5	8	95.7 ± 2.29
B6	8	94.8 ± 1.91
C1 (Control)	8	82.3 ± 6.59
C2	8	64.9 ± 5.94
C3	8	55.7 ± 6.08
C4	8	45.0 ± 3.04
C5	8	38.7 ± 5.17
C6	8	37.5 ± 4.14

The above results show that the extracting proportion of collagen of skin decreases as rats become older or have osteoporosis (the rats were ovariectomized) in the normal and control groups (i.e., Groups A and C). However, at the times of experimentation above and including 390±15 (i.e., subgroups 3 and above for each group), the CIBONNA group (Group B) had a statistically significant higher proportion of collagen extracted as compared to the normal and control groups ($p<.01$), and showed an increase in the proportion of collagen extracted over time.

C. Helps Build Bone: Bone Mineral Density of Rats

The thigh bones of each group of rats were obtained and soft tissues on the surface of the thigh bones were removed while the periosts were retained. The bone mineral density (BMD) of the thigh bones was measured by LUNAR dual energy X-ray bone density apparatus with small animal software (see Table 8).

Table 8. Analysis of Bone Mineral Content

Group	Sample	Time of Samples (week)	BMD (g/cm^2)
A1 (Normal)	30	0	0.269 ± 0.017
A2	30	12	0.266 ± 0.021
A3	30	24	0.241 ± 0.018
A4	30	36	0.224 ± 0.024
A5	30	48	0.168 ± 0.029
A6	30	60	0.149 ± 0.041
B1 (CIBONNA™)	30	0	0.238 ± 0.007
B2	30	12	0.261 ± 0.012
B3	30	24	0.267 ± 0.009
B4	30	36	0.269 ± 0.010
B5	30	48	0.272 ± 0.019
B6	30	60	0.274 ± 0.021
C1 (Control)	30	0	0.241 ± 0.013
C2	30	12	0.202 ± 0.010
C3	30	24	0.172 ± 0.019
C4	30	36	0.146 ± 0.011
C5	30	48	0.129 ± 0.017
C6	30	60	0.113 ± 0.014

As shown above, the BMD of the control group decreased rapidly with the aging of the rats, and the BMD of the normal group also decreased with the aging of the rats. In contrast, the BMD of the CIBONNA™ group steadily increased over time. At the times of experimentation above and including 390±15 (i.e., subgroups 3 and above for each group), the CIBONNA group (Group B) had a statistically significant BMD level as compared to the normal and control groups ($p<.01$).

D. Helps Build Bone: Biomechanical Tests of Rats

The thigh bone materials for the above bone mineral content analysis were utilized for biomechanical testing with a method pressure test at three points. The testing device used was a WG-1 type of electronic universal testing device (produced by Changchun Nonmetal Testing Device Factory). The biomechanical test results show that the biomedical performance of the rats was improved after taking CIBONNA™ (see Table 9). Specifically, the thigh bones of the CIBONNA group showed an increase in modulus elasticity, yield strength, destructibility strength, yield enthalpy and destructibility enthalpy over time, while the normal and control groups showed a decrease in these biomechanical measurements over time (see Table 9). At the times of experimentation above and including 390±15 (i.e., subgroups 3 and above for each group), the CIBONNA group (Group B) had statistically significant higher levels of the above mentioned biomechanical variables, as compared to the normal and control groups ($p<.01$).

Together, the findings in Tables 6, 7, 8, and 9 lead to several conclusions: (1) the BMC levels of the rats increased after they took CIBONNA™; (2) the proportion of the breaking time of collagen after the rats took CIBONNA™ decreased dramatically over time, while the breaking time of collagen for the normal and control groups increased over time; and (3) the extracting rate of collagen decreases as the BMC of the rats decreases.

III. Conclusion

CIBONNA™ can effectively help build strong bone in rats, as evidenced by increasing the BMD of rats having osteoporosis, as well as positive biomechanical test results for rats taking CIBONNA™. CIBONNA™ has also been shown to delay the aging process of rats based on (1) the breaking time of collagen fibers of the caudal tendon of rats after taking CIBONNA™ is much shorter than those who did not take CIBONNA™, and (2) rats taking CIBONNA™ had a statistically significant higher proportion of collagen extracted as compared to the normal and control groups and showed an increase in the proportion of collagen extracted over time.

Table 9. Biomechnical Analysis

Group	Sample	Modulus Elasticity *N/S*	Yield Strength *Newton (N)*	Destructibility Strength *Newton (N)*	Yield Enthalpy *KJ/mol*	Destructibility Enthalpy *KJ/mol*
A1	30	1.49 ± 0.095	8.81 ± 1.22	10.8 ± 1.21	6.85 ± 1.28	13.4 ± 1.37
A2	30	1.40 ± 0.011	8.59 ± 1.27	10.54± 1.26	6.55 ± 1.36	12.5 ± 1.46
A3	30	0.97 ± 0.099	6.43 ± 1.23	7.72 ± 1.22	4.63 ± 1.30	8.01± 1.40
A4	30	10.74± 0.13	5.23 ± 1.32	6.96 ± 1.30	3.52 ± 1.42	5.76 ± 1.55
A5	30	0.35 ± 0.18	2.75 ± 0.46	3.61 ± 1.38	1.58 ± 0.53	2.11 ± 0.84
A6	30	0.23 ± 0.21	2.20 ± 0.23	2.85 ± 0.59	1.25 ± 0.46	1.53 ± 0.52
B1	30	0.94 ± 0.066	6.19 ± 1.23	7.76 ± 1.08	4.39 ± 1.11	7.58 ± 1.10
B2	30	1.29 ± 0.075	7.91 ± 1.15	9.75 ± 1.14	6.12 ± 1.19	11.4 ± 1.26
B3	30	1.46 ± 0.10	8.78 ± 1.10	10.76 ± 1.10	6.69 ± 1.17	12.7 ± 1.16
B4	30	1.49 ± 0.14	8.75 ± 1.12	10.79 ± 1.22	6.81 ± 1.16	12.9 ± 1.21
B5	30	1.54 ± 0.21	9.12 ± 1.24	11.2 ± 1.34	7.22 ± 1.32	13.9 ± 1.46
B6	30	1.58 ± 0.24	9.24 ± 1.31	11.4 ± 1.46	7.19 ± 1.41	14.6 ± 1.41
C1	30	0.98 ± 0.14	6.50 ± 1.16	7.98 ± 1.15	4.58 ± 1.27	7.92 ± 1.18
C2	30	0.54 ± 0.091	4.16 ± 1.10	5.21 ± 1.11	2.55 ± 1.11	3.95 ± 1.21
C3	30	0.31 ± 0.071	2.78 ± 0.94	3.76 ± 1.23	1.71 ± 0.91	2.33 ± 0.70
C4	30	0.24 ± 0.047	2.14 ± 0.57	2.77 ± 0.92	1.23 ± 0.59	1.45 ± 0.61
C5	30	0.18 ± 0.052	1.76 ± 0.62	2.34 ± 0.63	0.87 ± 0.41	1.07 ± 0.36
C6	30	0.13 ± 0.055	1.46 ± 0.48	2.04 ± 0.56	0.47 ± 0.19	1.01 ± 0.31